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华支睾吸虫含串联重复序列的Cs22抗原蛋白的克隆及特征分析

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Molecular Cloning and Characterization of a Novel Clonorchis sinensis Antigenic Protein Containing Tandem Repeat Sequences

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摘要 目的 通过免疫学筛选华支睾吸虫成虫λ-ZAP cDNA表达文库和EST数据验证，寻找新的抗原基因，并对其免疫学特征进行初步鉴定。 方法 使用华支睾吸虫病患者混合血清筛选获得阳性噬菌体克隆，测序后与EST数据进行比对，逆转录PCR（RT-PCR）获得Cs22全长基因序列。PCR跳跃技术获得重复序列次数为2和3次的cDNA片段，将含成熟肽和串联重复序列部分分别克隆至原核表达质粒pET28a（＋）中，转化至宿主菌后，诱导表达获得重组蛋白rCs22-2r、rCs22-3r、rCs22M-2r和rCs22M-3r，使用组氨酸标签亲和纯化柱（Ni-NTA树脂）纯化。ELISA法鉴定重组蛋白rCs22-2r和rCs22-3r的免疫原性；检测35份华支睾吸虫虫卵粪检阳性的患者血清，15份日本血吸虫病、15份卫氏并殖吸虫病和13份猪囊尾蚴病的患者血清以及31份健康人血清，评价重组蛋白rCs22-2r和rCs22-3r的免疫学诊断价值。使用仅含串联重复多肽序列部分重组蛋白rCs22M-2r和rCs22M-3r，ELISA法分别检测华支睾吸虫病患者和健康人混合血清的特异性抗体，初步确定抗原决定簇位置。 结果 获得华支睾吸虫Cs22抗原基因的全长序列，其含有EQQDGDEEGMGGDGGRGKEKGKVEGEDGAGEQKEQA 36个氨基酸组成的串联重复多肽序列，共重复13次。生物信息学分析表明，Cs22蛋白为GPI锚定蛋白。ELISA结果显示，重组蛋白rCs22-2r和rCs22-3r具有一定的免疫原性；ELISA检测血清特异性抗体结果显示，华支睾吸虫病患者血清和健康人血清的阳性率均分别为45.7%（16/35）和3.2%（1/31），与日本血吸虫病和猪囊尾蚴病患者血清无交叉反应，与卫氏并殖吸虫病患者血清仅1份有交叉反应。ELISA法检测重组蛋白rCs22M-2r和rCs22M-3r结果显示，两者能区分华支睾吸虫病患者血清与健康人血清。 结论 获得华支睾吸虫Cs22抗原基因全长序列，其重组抗原蛋白具有一定的免疫学诊断价值，抗原决定簇位于串联重复多肽。

关键词： 华支睾吸虫 免疫学筛选 抗原 串联重复序列 GPI锚定蛋白

Abstract： Objective To find and clone new antigen genes from the λ-ZAP cDNA expression library of adult Clonorchis sinensis, and determine the immunological characteristics of the recombinant proteins. Methods The cDNA expression library of adult C. sinensis was screened by pooled sera of clonorchiasis patients. The sequences of the positive phage clones were compared with the sequences in EST database, and the full-length sequence of the gene（Cs22 gene） was obtained by RT-PCR. cDNA fragments containing 2 and 3 times tandem repeat sequences were generated by jumping PCR. The sequence encoding the mature peptide or the tandem repeat sequence was respectively cloned into the prokaryotic expression vector pET28a（＋）, and then transformed into E. coli Rosetta DE3 cells for expression. The recombinant proteins （rCs22-2r, rCs22-3r, rCs22M-2r, and rCs22M-3r） were purified by His-bind-resin（Ni-NTA） affinity chromatography. The immunogenicity of rCs22-2r and rCs22-3r was identified by ELISA. To evaluate the immunological diagnostic value of rCs22-2r and rCs22-3r, serum samples from 35 clonorchiasis patients, 31 healthy individuals, 15 schistosomiasis patients, 15 paragonimiasis westermani patients and 13 cysticercosis patients were examined by ELISA. To locate antigenic determinants, the pooled sera of clonorchiasis patients and healthy persons were analyzed for specific antibodies by ELISA with recombinant protein rCs22M-2r and rCs22M-3r containing the tandem repeat sequences. Results The full-length sequence of Cs22 antigen gene of C. sinensis was obtained. It contained 13 times tandem repeat sequences of EQQDGDEEGMGGDGGRGKEKGKVEGEDGAGEQKEQA. Bioinformatics analysis indicated that the protein（Cs22） belonged to GPI-anchored proteins family. The recombinant proteins rCs22-2r and rCs22-3r showed a certain level of immunogenicity. The positive rate by ELISA coated with the purified PrCs22-2r and PrCs22-3r for sera of clonorchiasis patients both were 45.7%（16/35）, and 3.2% （1/31） for those of healthy persons. There was no cross reaction with sera of schistosomiasis and cysticercosis patients. The cross reaction with sera of paragonimiasis westermani patients was 1/15. The recombinant proteins rCs22M-2r and rCs22M-3r which only contained tandem repeats were specifically recognized by pooled sera of clonorchiasis patients. Conclusion The Cs22 antigen gene of Clonorchis sinensis is obtained, and the recombinant proteins have certain diagnostic value. The antigenic determinant is located in tandem repeat sequences.

Keywords： Clonorchis sinensis; Immunological screening; Antigen; Tandem repeat sequence; GPI- anchored

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